Foliumzuur en enkele nieuwe antagonisten

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IX. SUMMARY.

As appears from the litterature survey in the beginning of this thesis, there was a great confusion on the vitamins of the Folic Acid (F.A.) group and on the growth factors active in the same field. Our review gives only the data which are necessary for consulting the detailed list of references (page 92—105).

Notwithstanding the numerous researches on F.A. and vitamin B_{12}, it is not yet known how they exert their haematopoietic action, especially with regard to their mutual relations. Also on the essential character of F.A. for haematopoiesis there has often been doubt since the isolation of vitamin B_{12}.

One of the methods used for getting an insight in the action of metabolites is the study of closely related compounds which act as antagonists.

The purpose of this research was to synthesize *antagonists to folic acid* and to examine their action on Streptococcus lactis R (S.L.R.).

First of all the preparation of pteroylglutamic acid or P.G.A. (the systematic name for folic acid) was tried by different ways. The first essays did not give the desired result; starting from hydroxymethylglyoxal and 2,4,5-triamino-6-hydroxy-pyrimidine we obtained a new pteridine, 2-amino-4-hydroxy-7-hydroxymethyl-pteridine; this pteridine was purified as diacetyl derivative.

The results of Karrer and Schwyzer's investigations [72, 452, 453, 454, 455]) on pteridines differ in several respects from those now recorded. More in accordance with our results were those obtained by Forrest and Walker [444]). With regard to a synthesis of 2-amino-4-hydroxy-6-hydroxymethyl-pteridine described by Karrer and Schwyzer, we could prove that, besides this product, at least two, but probably three other pteridines are formed, each in about the same yield.

The direct condensation of ethyl p-aminobenzoate and 2-amino-4-hydroxy-6-pteridylmethanal, which promised better results and already had let to the Schiff's base ethyl 9,10-dehydropteroate, was given up when a better new method appeared in the litterature.

The greatest difficulty is the isolation of the pure compound from the reaction mixture, which usually contains only a small percentage of P.G.A.

The isolation and purification described by Waller et coll. [81]) could not be reproduced. Addition of a definite quantity of calcium chloride to the alcaline solution of the crude product and neutralization of the mixture to $p_H = 7.0$ prevented the impurities to stay in colloidal solution as well as to occlude P.G.A. during their precipitation; this occlusion occurred when the concentration of $CaCl_2$ was too high. By submitting the precipitate formed at $p_H = 7.0$ several times to this purification process we succeeded in increasing the yield obtained by Waller to nearly the double value.

The two methods described in the litterature for preparing N-p-aminobenzoyl-1(+)glutamic acid (starting material for P.G.A.) were experimentally compared. Van der Scheer's method [465]) for reducing the corresponding nitro compound proved to be far superior to the newer method described by Ivănovics [450]). The yield could be increased to more than 80 %.

The substitution products of the nitro and amino acids, which served as starting materials of six P.G.A. derivatives, were prepared in the same way as the parent compounds; the isolation of the pure compounds demanded mostly special methods.

By reducing 2-chloro-4-nitrobenzoyl-glutamic acid with hydrogen and PtO_2 we got mixed crystals containing about one molecule of 4-aminobenzoyl-glutamic acid and two molecules of the 2-chloro derivative. Thus the chlorine has been partially split off. It is not impossible, though improbable, that the corresponding fluorine compound has been liable to the same fate.

The six following in the benzene nucleus substituted P.G.A. derivatives have been prepared by means of the method described above.

2′-chloro-pteroyl glutamic acid
2′-fluoro- „ „ „
2′-methyl- „ „ „
3′-methyl- „ „ „
2′-methoxy- „ „ „
3′-methoxy- „ „ „

Three of these compounds are competitive F.A. antagonists for Streptococcus lactis R.: 3′-methyl-P.G.A., 2′-methoxy-P.G.A. and 2′-methyl-P.G.A.; their antibacterial indices are, 100, 500 and 5000 respectively.

In this thesis the „antibacterial index" is defined as the quotient of the concentrations of antagonist and P.G.A., for which the growth of S.L.R. is just prohibited.

3′-Methoxy-P.G.A. has a feeble growth promoting action for S.L.R. 2′-Fluoro-P.G.A. promotes the growth of S.L.R. equally well

as P.G.A. itself; the identity of this compound, however, has not yet been wholly proved.

The influence of 2′-chloro-P.G.A., as growth promotor and as antagonist, is insignificant.

The microanalytical determination of nitrogen (Pregl-Dumas) was examined critically, especially with regard to the correction of the measured volume and the applicability in the case of hardly decomposing products.